NeuroToxicology 65 (2018) 22–27

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

The association between early-life relative telomere length and childhood neurodevelopment

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ARTICLE INFO

Article history:
Received 30 August 2017
Received in revised form 17 January 2018
Accepted 19 January 2018
Available online xxx

Keywords:
Children
Epidemiology
Cognition
Language

ABSTRACT

Purpose: To examine the association between telomere length and neurodevelopment in children.
Methods: We examined the relationship between relative telomere length (rTL) and neurodevelopmental outcomes at 9 and 30 months, and 5 years of age in children enrolled in the Seychelles Child Development Study Nutrition Cohort 1 (NC1). Relative telomere length was measured in cord blood and in child blood at age five. Multivariable linear regression examined associations between neurodevelopmental outcomes and rTL adjusting for relevant covariates.
Results: Mean rTL was 1.18 at birth and 0.71 at age five. Increased cord blood rTL was associated with better scores on two neurodevelopmental tests, the psychomotor developmental index ($\beta = 4.01$; 95% confidence interval (CI) = 0.17, 7.85) at age 30 months, and the Woodcock Johnson test of achievement letter-word score ($\beta = 2.88$; CI = 1.21–4.56) at age five. The Woodcock Johnson test of achievement letter-word score remained statistically significant after two outliers were excluded ($\beta = 2.83$; CI = 0.69, 4.97); the psychomotor developmental index did not ($\beta = 3.62$; CI = −1.28, 8.52). None of the neurodevelopmental outcomes at age five were associated with five-year rTL.
Conclusion: Although increased cord blood rTL was associated with better test scores for a few neurodevelopmental outcomes, this study found little consistent evidence of an association between rTL and neurodevelopment. Future studies with a larger sample size, longer follow-up, and other relevant biological markers (e.g. oxidative stress) are needed to clarify the role of rTL in neurodevelopment and its relevance as a potential surrogate measure for oxidative stress in the field of developmental neurotoxicity.

1. Introduction

Telomeres are non-coding, nucleoprotein complexes at the ends of eukaryotic chromosomes whose function is to preserve genomic integrity. Telomeres naturally shorten due to incomplete DNA replication during cellular divisions, otherwise known as cellular aging (Blackburn, 2001; McEachern et al., 2000). Telomeres eventually reach a critical point in length where they lose their protective functions and the cells stop dividing and enter either apoptosis or cellular senescence (Gisselsson et al., 2001; Murnane, 2006; Verdun and Karlseder, 2007). Therefore, telomere length can be considered to be a measure of our "biological" age as opposed to

Abbreviations: rTL, Relative telomere length; TL, Telomere length; SCDS, Seychelles Child Development Study; NC1, Nutritional Cohort 1; BSID-II, Bayley Scales of Infant Development-II; MDI, Mental Development Index; PDI, Psychomotor Development Index; SES, Socioeconomic status; GATB, General Aptitude-Test Battery; CANTAB, Cambridge Neuropsychological Test Automated Battery; HBB, Hemoglobin beta chain.

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https://doi.org/10.1016/j.neuro.2018.01.005
0161-813X/

our "chronological" age (Oeseburg et al., 2010). As a marker for cellular aging, telomere length (TL) is seen as a possible predictor of various age-related diseases, including cognitive decline. For example, telomere length has been associated with Alzheimer's disease, (Roberts et al., 2014; Zhan et al., 2015) as well as poor cognitive function in adults (Kingma et al., 2012; Valdes et al., 2010; Yaffe et al., 2011). Similarly, TL at young ages may be associated with cognition and neurodevelopment in early life or may be related to adult cognitive function in later life, likely as an indirect marker of other biological processes that may influence neurodevelopment such as oxidative stress (Epel et al., 2004; Houben et al., 2008; Von Zglinicki, 2000; von Zglinicki, 2002). TL may be considered a surrogate measure for underlying oxidative stress and inflammation and potentially a good biomarker of neurotoxicity, as has been indicated in previous literature (Epel et al., 2004; Houben et al., 2008; Von Zglinicki, 2000; von Zglinicki, 2002).

The relationship between TL and neurodevelopmental outcomes in children has received little attention. Only four studies to date have examined the association with neurobehavioral outcomes in children (Costa Dde et al., 2015; Henje Blom et al., 2015; Li et al., 2014; Wojcicki et al., 2015). Two of these studies have reported correlations between shorter TL and measures of depression and inattention in later childhood and adolescence (Costa Dde et al., 2015; Henje Blom et al., 2015). Other studies showed a shorter TL in young children with autism and defiant behavior (Li et al., 2014; Wojcicki et al., 2015). Adding to this limited literature, our study examines the association between relative telomere length (rTL) and cognitive development, including motor, language, memory and problem-solving skills. These are more subtle aspects of development that have not been previously assessed in relation with TL.

2. Methods

2.1. Study population

The Seychelles Child Development Study (SCDS) is a series of longitudinal observational studies that evaluate the development of children in Seychelles, and examine if mercury exposure during pregnancy (due to a high fish diet) is associated with child development. The present analysis used data from the Nutrition Cohort 1 (NC1) (Davidson et al., 2008), a cohort of 300 mothers that were enrolled in 2001 during their first trimester of pregnancy. The inclusion criteria included mothers at least 16 years of age, native born of Seychelles, and residing on Mahé. Exclusion criteria included infants with major congenital anomalies and twins. Research protocols were reviewed and approved by the Institutional Review Boards of the University of Rochester, the Ministry of Health in Republic of Seychelles and the Regional Ethics Committee, Lund University. The procedures followed were in accordance with the Helsinki Declaration, and all participants gave informed consent. The present study examined child telomere length at birth and at the time of examination at approximately age 5 years in relation to developmental outcomes assessed in the children at 9 months, 30 months, and 5 years of age.

2.2. Blood collection

Cord blood samples were collected immediately after delivery into EDTA-containing tubes, from which whole blood was obtained and stored at −80 °C until analysis. Similarly, children's venous non-fasting blood samples were collected in EDTA-containing tubes after completion of the 5-year developmental assessment, from which whole blood samples were obtained and stored at −80 °C.

2.3. Telomere length assessment

DNA was isolated from peripheral blood using the Qiagen DNA blood Midi kit (Qiagen, Hilden, Germany). Relative telomere length (rTL) was measured using real-time PCR (7900HT, Applied Biosystems, Foster City, CA, USA), as described previously (Ameer et al., 2016; Li et al., 2012). Briefly, master mixes were prepared, containing 0.5 U *Taq* Platina (Invitrogen, Carlsbad, CA, USA), 1 × PCR Buffer, 0.8 mM dNTPs, 1.75 mM $MgCl_2$, 0.3 mM SybrGreen (Invitrogen), 1 × Rox (Invitrogen), and either telomere primers (0.45 μM of each primer), or hemoglobin beta chain (*HBB*) primers (0.45 μM for each primer). Five microliters of sample DNA (3 ng/μl) was added to each reaction resulting in a final volume of 20 μl. A standard curve, a reference DNA, and a negative control were also included in each run, and all samples, standards, and controls were run in triplicate. The relative length of the telomeres was obtained through calculating the ratio (T/S) of the telomere repeat product to a single-copy gene product (S, here *HBB*) for each individual, by the formula $T/S = 2^{-DCt}$, where $\Delta Ct = Ct_{telomere} - Ct_{HBB}$. This ratio was then compared with the ratio of a reference DNA. The telomere length ratio is an arbitrary value. Relative telomere length was measured at birth from cord blood and again in blood collected at five years of age.

2.4. Neurodevelopmental assessment

We analyzed data from the Bayley Scales of Infant Development-II (BSID-II), a well-standardized measure of infant cognition and development that was administered at ages 9 and 30 months. The BSID-II yielded two endpoints: the mental developmental index (MDI) and psychomotor developmental index (PDI) (Davidson et al., 2008). We also examined the following developmental tests at five years of age: finger tapping (dominant and non-dominant hand), the Preschool Language Scale (total language score, verbal ability, and auditory comprehension), the Woodcock-Johnson Scholastic Achievement Test (letter-word recognition and applied problems), the Kaufman Brief Intelligence Test (verbal knowledge, matrices), and the Child Behavior Checklist (Strain et al., 2012).

2.5. Covariates

As in previous studies of this cohort, covariates were selected *a priori* based on their known association with developmental outcomes (Strain et al., 2008; Strain et al., 2012). Covariates included: child sex, birth weight, age of child at testing, Hollingshead socioeconomic status (SES) at birth, maternal IQ, maternal age at birth of child, family status (i.e. whether or not both parents resided with the child) at 9 months, and home environment at birth. Smoking was not included as a covariate because only eight mothers reported smoking during pregnancy.

2.6. Statistical analysis

Descriptive statistics for relevant sample characteristics were calculated, including the mean, median, and standard deviation for all continuous variables, the proportions for categorical variables, and the distribution of cord blood rTL, 5-year rTL, and all neurodevelopment outcomes. We examined correlation coefficients for the association between cord rTL, 5-year r-TL, and change in rTL from birth to 5-years of age. Cord rTL and five-year rTL were poorly correlated (spearman $r = 0.26$, $p = 0.0007$; pearson $r = 0.14$, $p = 0.067$), whereas cord rTL and change in rTL were highly correlated (spearman $r = -0.90$, $p < 0.0001$; pearson $r = -0.98$, $p < 0.0001$). Therefore, the change in rTL was not further investigated as it did not contribute additional statistical